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Bisphenol A removal from a plastic industry wastewater by *Dracaena sanderiana* ~~/~~ endophytic bacteria and *Bacillus cereus* NI

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Abstract

Understanding the significance of plant-endophytic bacteria for bisphenol A (BPA) removal is of importance for any application of organic pollutant phytoremediation. However, there is no information on the role of plant-endophytic bacteria for ~~in~~ plastic industry wastewater treatment. In this research, *Dracaena sanderiana* with ~~/~~ endophytic *Pantoea dispersa* showed higher BPA removal than un ~~non~~ inoculated plants at 89.54 ± 0.88% and 79.08 ± 1.20%, respectively. Quantitative Real-Time PCR (qPCR) showed that *P. dispersa* increased from 3.93×10^7 to 8.80×10^7 16S rRNA gene copy number in root tissues from day 0 to day 5 of the treatment period which indicated that it could assist the plant in removing BPA during the treatment process. The wastewater characteristics for ~~of~~ each condition, particularly

What condition?

biochemical oxygen demand (BOD), significantly decreased due to activities of the microorganisms. Furthermore, an indigenous bacterial strain with BPA-removal ability which was isolated from the wastewater, *Bacillus cereus* NI, could remove BPA in the presence of high total dissolved solids (TDS) and alkaline pH of the wastewater. In addition, combinations of plant-endophytic bacteria and *B. cereus* NI showed the highest BPA removal efficiency when compared with other conditions. This work suggests that ~~using~~ *D. sanderiana* plants could be used as a secondary process in a wastewater treatment system and *B. cereus* NI could also be applied for BPA removal from wastewaters with ~~of~~ high TDS, alkalinity and salinity ~~wastewater~~.

Keywords Bisphenol A, *Dracaena sanderiana,* Endophytic bacteria, *Bacillus cereus*, Plastic industry wastewater

1 Introduction

The release of a variety of organic pollutants by an increasing number of industries worldwide causes environmental problems which can lead to adverse effects on living systems. Many of these pollutants are recognized as Endocrine Disruptor Chemicals (EDC) because of their ability to interfere with the human endocrine system (Staples et al. 2002; Carlisle et al. 2009; Fu et al. 2010). Among EDC, ~~B~~bisphenol A (BPA) is one of the most abundant in the aquatic environment, and can affect surface and ground water systems ~~and the surrounding land and drinking waters~~ (Carlisle et al. 2009). BPA is a compound which is used in the manufacture of plastics, epoxy resins, and polycarbonates ~~BPA~~ and is also used in a number of other products such as adhesives, building materials, flame retardants, and lacquer coatings on food cans (Yamamoto et al. 2001; Belfroid et al. 2002). Low concentrations of BPA, within the range of 0.1 to 10 μM can show estrogenic and mutagenic effects in humans and the range of 0.04 to 0.4 μM can show acute toxicity toward aquatic organisms such as algae, invertebrates, and fish (Zhang et al. 2013).

BPA is increasingly detected in ~~but~~ the environment (Yamamoto et al. 2001; Belfroid et al. 2002; Fu et al. 2010; Saiyood et al. 2010; Zhang et al. 2013). In Thailand, the BPA concentration in the effluent from five wastewater treatment plants (Dindaeng, Rattanakosin, Chong NonSi, Thungkru and Nong Khaem) in Bangkok ranged between 0.251×10^{-15} μM and 1.125×10^{-15} μM (Pookpoosa et al. 2014). In the Nan river, Phisanulok, Thailand, the level of BPA ranged from 0.153×10^{-15} to 6.80×10^{-15} μM. These levels of BPA are higher than those found in many others countries such as

South Korea, Australia, China and the USA (Deemoon et al. 2016). Due to BPA dissemination and potential harmful effects, the development of an efficient system for removal is therefore necessary.

Although several physical and chemical methods can be used to remove BPA such as photodegradation by TiO_2 and UV (Neamtu et al. 2006; Wang et al. 2009), ozonation (Deborde et al. 2008), membrane filtration (Zhang et al. 2006), and Fenton oxidation and peroxidation (Mohapatra et al. 2010), these cleanup technologies have significant engineering costs and ~~provide~~ produce some secondary wastes as by-products (Suyamud et al. 2018). Biological treatment using phytoremediation bio technology which encompasses microbial and plant–mediated degradation, has ~~been~~ proved to be a promising method in recent years, especially, the application of functional plant endophytic bacteria ~~within plants~~ ~~h~~as a potential strategy because of (1) ~~the~~ enhancement of the plant's phytoremediation, (2) ~~gives~~ new insights into novel protocols to improve phytoremediation, and (3) a sustainable approach for decontamination of organic pollutants (Afzal et al. 2014). ~~Many~~ Much research ~~are~~ has ~~also~~ proposed the advantages of plant-endophytic bacteria to remove organic pollutants. For example, the exploitation of *Dracaena sanderiana* - endophytic bacteria has ~~contributed~~ provided a strategy for enhancing degradation rates, alleviation of stress-mediated impacts of BPA, enhancing phytohormone production, e.g. indole-3-acetic acid (IAA), and ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity and protection of host plants by controlling intracellular reactive oxygen species (ROS) (Suyamud et al. 2018). The ~~inoculating~~ functional endophytic bacterial strain *Achromobacter xylosoxidans* F3B ha~~s~~d ~~a~~ the potential to improve ~~the~~ aromatic pollutants removal (Ho et al. 2013), ~~and~~ while fenpropathrin degradation was also enhanced by *Spirodela polyrrhiza* and its endophyte bacteria (Xu et al. 2015). Moreover, the endophytic bacterial community ~~in planta~~ played a major role in removal of organic substances and improving growth of the halophyte *Juncus acutus* ~~growth~~ (Syranidou et al. 2017). Endophytic bacteria ~~itself also~~ exhibit~~ed~~s a remarkable capability to ~~uptake~~ accumulate and metabolize organic compounds ~~and~~ while plant metabolism of organic pollutant leads to the formation of biologically active metabolites (Carter et al. 2018; Suyamud et al. 2018). Although cultivable endophytic bacteria with degradation potential are highly promising candidates for bioaugmentation strategies, the application of such bacteria for wastewater treatment are unreported. Wastewaters can support ~~a~~ large and divers~~ified~~e bacterial communities, and specific degraders in the entire microcosm must also play a major role in degradation. In the wastewater of BPA-using industries, ~~are found~~ BPA-degrading bacteria are found which may be useful for BPA removal. The use of bacterial strains isolated from polluted sites as a practical system~~s~~ for removing BPA is promising. Therefore, ~~the~~ bacterial strains from a plastic industry wastewater ~~was~~ were also isolated for the treatment of BPA.

This study aims to gain a greater insight into BPA removal from a plastic industry wastewater by inoculating *Dracaena sanderiana* plants with BPA-degrading endophytic bacteria which can promote plant growth and utilize BPA for their growth. From our previous study, *D. sanderiana* inoculated with the root endophyte *Pantoea dispersa* showed the highest BPA removal efficiency of 92.32 ± 1.23 % at an initial BPA concentration of 20 µM in a synthetic solution within 21 days of the experimental period (Suyamud et al. 2018). *P. dispersa* can produce IAA, utilize ACC deaminase for nitrogen sources and protect the plants by lowering the level of ROS from BPA stress. *D. sanderiana* (ribbon plant) ~~plant~~ was selected for BPA removal in this study because it showed high BPA removal ability reaching up to 85% of the initial concentration of 20 µM and tolerance to BPA toxicity up to the 80 µM level (Saiyood et al., 2010). Thus, the role of plant-endophytic bacteria has been further studied and applied to wastewater from the plastic industry. Sustainability of plant-endophytic bacteria in the wastewater was assessed and ~~the~~ wastewater physico-chemical characteristics were determined after the treatment process. Furthermore, combinations of *D. sanderiana*-endophytic bacteria with *Bacillus cereus* NI, which was isolated from ~~the~~ plastic industry wastewater, ~~was~~ were compared in order to select an appropriate method for BPA removal in different kinds of treatment process~~es~~.

2 Materials and methods

2.1 Chemicals and Growth Medi~~um~~a

BPA (>99%) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Acetonitrile (HPLC grade) was purchased from Sigma-Aldrich. Mineral salts medium (MSM) (pH 6.8 ± 0.2) was used as an isolation and cultivation media. The components of the medium consisted of (g L^{-1}): Na_2HPO_4 1.4196 g, KH_2PO_4 1.3609 g, $MgSO_4.7H_2O$ 0.0985 g, $CaCl_2.2H_2O$ 0.0059 g, H_3BO_3 0.116 g, $Fe_3SO_4.7H_2O$ 0.278 g, $ZnSO_4.7H_2O$ 0.115 g, $MnSO_4.H_2O$ 0.169 g, $CuSO_4.H_2O$ 0.038 g, $CoCl_2.6H_2O$ 0.024 g, MoO_3 0.010 g. Yeast extract, Luria-Bertani (LB) medium (pH 7.0) and agar 15 g L^{-1} were also ~~prepared~~ used for this experiment.

city? country?

g L^{-1}? what?

milliQ H_2O

what medium? concentration?

what kind?

2.2 Wastewater Sampling

Wastewater before entry into a wastewater treatment system was collected from a plastic producing industry in Thailand, and ~~The wastewater was~~ kept at 4°C until used. The BPA concentration was ~~detected~~ measured at 10.43 µM or 2.38 ± 0.19 mg L^{-1}, ~~measured~~ using high performance liquid chromatography (HPLC). BPA analysis followed the method

city?

of Suyamud et al. (2018)~~. More details are provided in the~~ (see supplementary data). All experiments were performed in triplicate.

2.3 Plant Preparation for BPA Treatment

D. sanderiana plants were selected as the model plant in this study. Two-month old plants were cultured according to ~~the method of~~ Suyamud et al. (2018). Briefly, the plants were cultivated in a hydroponic system using ~~a~~ 2-L glass jars containing half-strength Hoagland's nutrient solution until their roots were fully grown ~~for~~ after 1 month. ~~Then,~~ The plants were then placed under cool white fluorescence lamps (1200-1300 lux) for a 12 h photoperiod at room temperature (28-30 °C). The roots and stems were washed several times with distilled water before use for BPA treatment.

sterile?

2.4 Endophytic Bacteria Culture Preparation

An endophytic strain of *P. dispersa,* as used in our previous study (Suyamud et al. 2018), was grown in LB medium at 30 °C on a rotary shaker at 150 rpm to an approximate cell concentration of 10^9 CFU mL^{-1}. The bacterial cells were collected by centrifugation at 2,415×g for 10 min. The collected cells were washed three times in sterile 10 mM $MgSO_4$ solution and suspended in sterile distilled water to obtain an inoculum density of 10^9 CFU mL^{-1}. Subsequently, 10% (v/v) of inoculum was added to each ~~pot~~ 0.8 L glass jar containing 500 mL ~~of~~ sterile distilled water. Roots of the plants were submerged in the inoculum solution for 5 min in order to achieve ~~appropriate~~ adequate inoculation.

2.5 Sustainability of Plant-Endophytic Bacteria in Wastewater Assessed by Quantitative Real-Time PCR

Plant root samples for each treatment at the beginning and at the end of the experiment were collected for DNA extraction using a E.Z.N.A. Soil DNA Kit (OMEGA bio-tek, USA) following the manufacturer's instructions. Before DNA extraction, root samples were incubated in sterile phosphate-buffered saline (pH 7.0) for 15 min. ~~Then~~ Plant roots were then surfaced sterilized using 2.5% NaOCl for 30 min, then washed three times with sterile distilled water. DNA integrity was verified using a nanodrop spectrophotometer and was stored at -20 °C until further use as the qPCR template. PCR amplification of 16S rRNA genes from the endophytic bacterium *P. dispersa* was performed using the universal primers of 785F and 907R. PCR amplicons were sequenced by Macrogen (Seoul, Korea). The specificity of each designed primer was evaluated using Primer-BLAST (NCBI) (Khaksar et al. 2016a). The sequences of the 16S rRNA specific primers were as follows: forward primer, GCATTTCACCGCTACACC; reverse primer, GCAGGCGGTCTGTTAAGTCA. The method of strain-specific qPCR followed the method of Suyamud et al. (2018). More details are provided in the supplementary data.

2.6 Enrichment and Isolation of BPA-Removing Bacteria from ~~the~~ Wastewater

The industrial wastewater was used as the ~~a~~ source for bacterial enrichment and isolation. One milliliter of the wastewater was added to mineral salts medium (MSM) containing 100 mg/L BPA at 30 ± 2 °C on a rotary shaker at 150 rpm for one week. After that, 1% (v/v) of the suspension was spread on fresh MM agar plates containing ~~with~~ 100 mg L^{-1} BPA for bacterial isolation. To ensure purity of the bacterial isolate, selected bacterial colonies were streaked on MSM agar plates three times.

2.7 Identification and Characterization of *Bacillus cereus* NI

The methods used to identify and characterize the selected bacteria *B. cereus* NI included colony morphology, Gram-staining and 16S ribosomal RNA (rRNA) gene sequence~~s~~ analysis. The latter ~~analysis~~ used universal 785F and 907R primers for DNA amplification supplied by Macrogen (Seoul, Korea). The obtained sequence was checked and compared with the National Center for Biotechnology Information (NCBI) database using the BLAST algorithm with the BLAST server. The phylogenetic tree was conducted using ~~by~~ MEGA 7.0 software and the ~~using~~ neighb~~o~~ur-joining method with a bootstrap value of 1000 replicates.

2.8 Growth and BPA removal Efficiency of *Bacillus cereus* NI

One loop of ~~the~~ *B. cereus* NI was inoculated into 5 mL LB medium. This cell starter culture, 3% (v/v), was then inoculated into mineral salts medium containing 0.1% (w/v) yeast extract and shaken at 200 rpm at room temperature for 8 h. The bacterial cells were collected by centrifugation at 4,180 g for 20 min, and ~~Then, harvested cells were~~ washed twice with 0.85% (w/v) sterile sodium chloride. The harvested culture 20% (v/v) was inoculated into wastewater containing BPA in 250 mL shake flasks at 30 ± 2 °C, 150 rpm for 48 h. The initial inoculum concentration was 10^9 CFU mL^{-1}. BPA remaining in solution was analyzed using HPLC to determine the BPA removal efficiency. In addition, ~~the~~ samples were measured for bacterial growth using OD_{600}.

2.9 BPA Removal by Plant-Endophytic Bacteria and *Bacillus cereus* NI

D. sanderiana-endophytic bacteria were tested for BPA removal during 5 days of the experimental period. After preparation of a 10% endophytic bacterial inoculum, experiments were carried out in triplicate under the following conditions: (1) sterile wastewater (500 mL); (2) wastewater (500 mL) + ~~non~~ un inoculated plants; and (3) wastewater (500 mL) + inoculated plants. Three plants per pot were used in this study. In addition, the BPA removal efficiency of the plant-endophytic bacteria was compared with *B. cereus* NI in the same volume of wastewater. A 10% Inoculum ~~10%~~ of *B. cereus* NI was prepared ~~to~~ for use in this study. Experiments were carried out in triplicate under the following conditions: (1) wastewater (500 mL); (2) wastewater (500 mL) + *B. cereus* NI; (3) wastewater (500 mL) + inoculated plants; (4) wastewater (500 mL) + *B. cereus* NI + inoculated plants. Water samples were collected at time intervals and kept at 4 °C until the analyses were conducted. The BPA concentration was analyzed by HPLC.

2.10 Analysis of Wastewater Characteristics

Wastewater characteristics before and after treatments were evaluated following standard methods for examination of water and wastewater published by the American Public Health Association (APHA) (APHA, 2005). pH was measured ~~by~~ using a pH meter (F2FiveGO, Mettler Toledo), chemical oxygen demand (COD) was analyzed by the potassium dichromate digestion method, biochemical oxygen demand (BOD) was analyzed by the azide modification method, total dissolved solids (TDS), salinity and conductivity were measured using an electrical conductivity meter (YSI, model 3200, USA). All measurements were performed in triplicate.

2.11 Statistical Analysis

Statistical analysis was performed using the SPSS program version 22.0. Levene tests were conducted for assessing ~~the~~ normal distribution. One-way ANOVA was performed followed by post-hoc analysis, and Tukey's honestly significant difference test (HSD) ($p<0.05$) used to assess different groups of ~~the~~ data.

3 Results and discussion

3.1 BPA Removal by Plant-Endophytic Bacteria

The plant endophytic-bacterium *P. dispersa* and its native host *D. sanderiana* were evaluated for the ability ~~and feasibility~~ to treat industrial wastewater containing BPA. The results showed that inoculated plants with the endophyte exhibited the highest BPA removal efficiency at 89.54 ± 0.88 % followed by the ~~non~~ un inoculated plants at 79.08 ±

1.20% and the sterile wastewater at 9.61 ± 1.27% after 5 days of treatment (Fig. 1). Each treatment was performed under hydroponic conditions. In case of the sterile wastewater, it was found that loss of BPA from the initial concentration ~~which~~ was caused by temperature ~~under~~ from the fluorescence light conditions (31-32 °C). Light and temperature can ~~create~~ induce a high degree of BPA degradation (Kang et al. 2005; Eio et al. 2014). These results demonstrated that endophytic *P. dispersa* had a significant positive effect on BPA removal ~~in~~ from the wastewater. In our previous study, endophytic *P. dispersa* itself could utilize BPA for growth and possessed a BPA removal efficiency of 68.94 ± 10.04 % after 2 days in a mineral salts medium supplemented with a BPA concentration of 20 μM (Suyamud et al. 2018). Furthermore, endophytic *P. dispersa* could confer protection to ~~its~~ the host plant against ~~BPA~~ stress from BPA and other compounds in the wastewater. ~~The~~ Plant growth promoting (PGP) endophytic bacteria can provide phytohormones, such as IAA which controls and lowers ROS accumulation (Khaksar et al. 2016a). Moreover, PGP endophytic bacteria possess~~ed~~ ACC deaminase activity which ~~could~~ can cleave ACC to ammonia and α-ketobutyrate. The lowering of ACC levels could be controlled by ethylene levels, which at high concentration could inhibit growth and stress the plant because ACC is a precursor of ethylene biosynthesis in plants (Adams and Yang 1979; Mayak et al. 2004; Khaksar et al. 2016a).

To investigate whether endophytic *P. dispersa* could assist in phytoremediation by *D. sanderiana* during the treatment period in the wastewater system, the degree of bacterial colonization of the plant roots was ~~performed by~~ assessed using quantitative real-time PCR (qPCR). The results indicated a significant increase in 16S rRNA gene copy numbers from $3.93 \times 10^7 \pm 1.27 \times 10^6$ to $8.80 \times 10^7 \pm 5.07 \times 10^6$ from day 1 to day 5, respectively, while ~~non~~ uninoculated plants had 16S rRNA gene copies at $7.34 \times 10^4 \pm 6.36 \times 10^3$ ($P<0.05$). This result confirmed sustainability of the population of the endophyte within the endophyte-inoculated plants over the treatment process. The endophytic bacteria could increase the performance of phytoremediation by enhancing BPA removal efficiency through promoting plant growth and BPA utilization. This result agrees with previous reports indicating that inoculation by endophytic bacteria is associated with plant-enhanced phytoremediation of toxic environmental contaminants (Babu et al. 2013; Afzal et al. 2014; Xu et al. 2015; Khaksar et al. 2016b).

3.2 Wastewater Characteristics in Relation to BPA Removal by Plant-Endophytic Bacteria

Industrial wastewater from plastics factories generally contain BPA. Discharges of such wastewaters are of primary concern due to their toxic effects (Badiefar et al. 2015). The physical and chemical characteristics of the initial wastewater were odour (no odour), colour (no colour), pH (9.32), COD (809 mg L^{-1}), BOD (107 mg L^{-1}), TDS (56,379 mg L^{-1}), conductivity (48.21 $\mu S\ cm^{-1}$), and salinity (46 ppt) with an initial BPA concentration of 10.43 μM. The characteristics of the wastewater before and after treatment are shown in Table 1. According to the results, the initial wastewater contained high TDS ~~and~~ possessed an alkaline pH. In this case, the major inorganic components of the wastewater were Cl 68.20 %, Na_2O 37.60 %, SO_2 7.24 %, P_2O_5 2.38 %, and others 0.43 % (K_2O, SiO_2, CaO, Fe_2O, Al_2O_3, CuO, and Br) (Saiyood et al. 2012). The wastewater ~~which~~ used in this study was from the same source ~~with~~ as in Saiyood ~~experiment.~~ et al. (2012). After 5 days of treatment, the pH of the wastewater gradually ~~reduced~~ decreased, particularly in the wastewater containing plants (Table 1) while the pH ~~was reduced~~ fell from 9.32 to 8.84. The decreasing ~~in~~ pH resulted from the secretion of extracellular polysaccharide (EP) and organic acids from plant roots. The plant roots ~~could~~ secreted EP under hydroponic conditions which was a result of the defence mechanisms of the plant (Saiyood et al. 2010; Suyamud et al. 2018). Furthermore, ~~the~~ the ✓ OK plant roots could also secrete organic acids (Syranidou et al. 2017). Root exudates can participate in mobilizing metal micronutrients or compete for binding sites with anionic species and also serve as a substrate for co-metabolism or stimulation of the degradation of organic pollutants (Bais et al. 2006; Wenzel et al. 2009).

Interestingly, the BOD of the wastewater dramatically decreased from 107 mg L^{-1} for the initial wastewater to 30 mg L^{-1} in case of the wastewater containing inoculated plants (Table. 1). This ~~result~~ indicated that the inoculated plants with bacteria or adding plants with natural bacteria to the wastewater system could decrease the BOD. In addition, the BOD of wastewater after 5 days was also reduced to 45 mg L^{-1} due to the natural microbial community in the wastewater having a major role in degrading the organic compounds (Goldman and Alexander 1983). Other characteristics of the treated wastewater were also decreased from those of the initial wastewater. However, ~~the~~ wastewater characteristics for ~~the~~ inoculated plants ~~and non~~ un inoculated plants were not significantly different in terms of BOD, COD, TDS, pH, conductivity and salinity ($p<0.05$) (Table 1) but significantly different in BPA removal (Fig. 1). ~~During~~ Over 5 days of treatment, endophytic bacteria which were inoculated by submersion could partially move into the internal root tissues. Endophytic bacteria could transfer from the roots to the shoots of the plant ~~host~~ which ~~will~~ would favour BPA removal by the host through plant growth-promoting traits, such as IAA production, ACC deaminase activity or reduction of ethylene levels (Prum et al. 2018). Therefore, *D. sanderiana* plants and their endophytic bacteria have demonstrated their potential application for phytoremediation of BPA in industrial wastewater.

3.3 Characteristic s of *Bacillus cereus* NI

After a one-week enrichment of the wastewater with BPA, bacterial colonies with clearly defined morphologies were obtained on BPA-selective plates. A bacterial isolate, strain NI, was specifically chosen based on its ~~highest~~ growth ~~efficiency~~ and tolerance to BPA at high concentrations. The selected strain NI ~~is~~ was a Gram-positive, spore forming and rod-shaped bacterium. ~~The~~ Colonies grown on LB agar were circular, raised with undulate margins and non-pigmented. The sequencing of 16S rRNA gene from this organism was achieved using 785F and 907R primers. The sequence was compared to those in the NCBI database using the BLAST algorithm with the BLAST server. The results indicated that this strain ha~~s~~d been deposited in the GenBank database under the accession number ~~of~~ # MH266620. The phylogenetic tree of this strain is shown in Figure 2. The 16S rRNA gene sequence comparisons show this organism is a strain of *Bacillus cereus*. This ~~result~~ agrees with a previous report showing that *B. cereus* could dissipate BPA in a hydroponic system (Saiyood et al. 2010). Previously, BPA removal and/or degradation has been shown in several bacterial genera such as *Sphingomonas bisphenolicum* strain AO1, *Achromobacter xylosoxidans* strain B-16, *Pseudomonas aeruginosa*, and *Cupriavidus basilensis* strain SBUG 290 (Kang et al. 2002; Sasaki et al. 2005a; Sasaki et al. 2005b; Mita et al. 2015; Zühlke et al. 2017).

Results for *B. cereus* NI showed that it could remove BPA ~~in~~ from 500 mL ~~of~~ wastewater with an inoculum of 10% (v/v) ~~at~~ 88.90 ± 0.43% (Fig. 4). In addition, *B. cereus* NI completely removed BPA ~~in~~ from 100 mL ~~of the~~ wastewater with an inoculum of 3% (v/v) within 48 h in the wastewater containing mineral salts medium (Fig. 3). This ~~result~~ indicated that *B. cereus* NI could utilize BPA for growth and tolerate the physico-chemical conditions ~~in~~ of the wastewater, particularly with additional nutrients. The addition of nutrients ~~could~~ enhanced growth and BPA removal by several bacterial species (Yamanaka et al. 2007)) such as *Cupriavidus basilensis* JF1(Fischer et al. 2010), *Pseudomonas* sp. strain KU1, KU2, *Bacillus* sp. strain KU3 (Kamaraj et al. 2014), *Bacillus thuringiensis* and *P. dispersa* (Suyamud et al. 2018). In addition, the presence of a more available carbon source in the wastewater could increase cell growth and reduce the level of BPA toxicity (Zhang et al. 2007). Thus, *B. cereus* NI could be used to treat BPA in wastewater by adding appropriate nutrients and this organism can also tolerate high TDS and an alkaline environment. ~~This indicates~~ *B. cereus* strain NI is therefore a promising ~~bacterium~~ organism for BPA removal from industrial wastewaters contaminated with BPA.

3.4 Comparison of BPA Removal Efficiency between Plant-Endophytic Bacteria and *Bacillus cereus* NI

Plant-endophytic bacteria and *B. cereus* NI were compared in order to select the system that is appropriate for BPA removal in the various kinds of treatment ~~process~~ after 5 days of the experiment. The results showed that wastewater containing inoculated plants and *B. cereus* NI displayed the highest BPA removal at 99.23 ± 0.10% while values for those that contained only inoculated plants or *B. cereus* NI alone were 97.75 ± 0.20% and 88.90 ± 0.43%, respectively (Fig. 4). The efficiencies of BPA removal therefore varied with different conditions.

It appears that the metabolism of inoculated plants and bioaugmented bacteria served as the main route for BPA removal. The mechanism of BPA removal by plant-endophytic bacteria in the wastewater is ~~complicated~~ complex because of both biotic and abiotic factors resulting from the effects of the natural microbial community and other components. The plant itself can secrete organic compounds such as monocarboxylic acids (formic, lactic acids) and di-, and tricarboxylic acids (oxalic, malonic and tartaric acids) which ~~are~~ have been found in the presence of BPA (Syranidou et al. 2017). In addition, extracellular polysaccharide (EP) can also be secreted by plants (Zimmermann et al. 1994; Pimienta-Barrios and Nobel 1998; Saiyood et al. 2010). Organic compound secretion could provide additional nutrients for growth of *B. cereus* NI which is present in the wastewater. These results provide convincing evidence that BPA could be also reduced by this process. The inoculated plants displayed a lower BPA removal efficiency at 97.75 ± 0.20 % compared to the inoculated plants with *B. cereus* NI. The higher removal efficiency of the inoculated plants indicated that the endophytic bacteria improved plant activity in the wastewater in two different ways: 1) directly by producing plant growth beneficial substances, e.g. ~~production of~~ phytohormones and ACC deaminase; and 2) indirectly through controlling plant phytotoxicity or by inducing some systemic resistance against pollutant stress, e.g. reduc~~e~~tion of metal phytotoxicity via extracellular precipitation (Babu et al. 2015), intracellular accumulation and sequestration (Shin et al. 2012), biotransformation to less- or nontoxic forms (Zhu et al. 2014), and adsorption (Guo et al. 2010; Luo et al. 2011). In addition, endophytic bacteria can produce substances that may effectively limit ~~the~~ phytopathogens by producing antibiotics, siderophores, a variety of hydrolytic enzymes and antimicrobial volatile organic compounds (Sheoran et al., 2015). Endophytic *P. dispersa* is also reported to synthesize antibiotic metabolites which can ~~assist~~ enhance the resistance of plants against pathogens (Zhang and Birch 1997; Chauhan et al. 2015; Ma et al. 2016). However, the hydrophobicity of BPA means it can be ~~absorb~~ absorbed by root and/or bacterial biomass, ~~then~~ leading to uptake through plant xylem ~~resulted in the~~ and a reduction of BPA concentration in solution. Organic pollutants could a~~lso uptake~~ be accumulated and transformed in the plant cells. Plant metabolism can lead~~s~~ to the formation of biologically active metabolites (Carter et al. 2018).

Although the treatments that contained the plants had the highest removal efficiency, plants did not survive for a long time in the wastewater after treatment for 5 days ~~resulted from~~ because of a phytotoxic effect of the wastewater ~~from a plastic industry include~~ which contained high amounts of Cl^- and Na^+ as well as other compounds (Prokop'ev 2001; Saiyood et al. 2013). It should be noted that for wastewater treatment using plants, the ability of plants to tolerate, survive and grow under stressful conditions is ~~also~~ an important feature for sustainable operation (Saiyood et al. 2013). However, it is possible to use plants as a secondary treatment if the wastewater does not contain high amounts of salt. This ~~study~~ work has provide~~s~~d convincing evidence that using plants, bacteria or plants coupled with bacteria to remediate BPA in the wastewater environment is a potentially effective alternative treatment technology which could be ~~selected~~ used depending on the characteristics of the wastewater treatment process.

4 Conclusions

This study has reported on the application of *Dracaena*-endophytic *Pantoea dispersa* and *Bacillus cereus* NI isolated from a plastic industry wastewater on BPA removal. Inoculated plants with endophytic bacteria showed a high BPA removal compared with ~~non-~~ un inoculated plants. The results of qPCR showed that *P. dispersa* could assist the plant in removing BPA over ~~the~~ 5 days of treatment ~~process~~. In the wastewater system, plants could reduce the alkalinity of the wastewater leading to enhanced BPA removal. The wastewater characteristics ~~of~~ for each condition, particularly BOD, ~~were~~ significantly decreased from the initial concentration due to the natural bacterial community. Furthermore, *B. cereus* NI could grow and remove BPA present in the wastewater and could also tolerate high TDS and alkaline conditions, particularly with addition of nutrients. In addition, combinations of plant-endophytic bacteria and *B. cereus* NI showed the highest BPA removal efficiency. This ~~study~~ suggests that *D. sanderiana* plants with their endophytes could be used as a secondary treatment for wastewater. Furthermore, *B. cereus* NI could be used for BPA removal in wastewater containing high TDS and salinity. However, the mechanism of BPA degradation in plant should be further investigated.

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Compliance with ethical standards

This ~~article~~ work ~~does~~ did not contain any studies with human participants or animals ~~performed by any of the authors~~.

Conflict of interest

The authors ~~declare that they~~ have no conflict of interest.

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Figure captions

Figure 1 BPA removal by plant-endophytic bacteria in a plastic industry wastewater at various conditions; (□) sterile wastewater, (▨) wastewater + plant, and (■) wastewater + plant + endophytic bacteria. Bars indicate the mean ± SD of three replicates.

Figure 2 16S rRNA sequence-based phylogenetic position of *B. cereus* NI.

Figure 3 Cell density (A) and BPA removal efficiency (B) of *B. cereus* NI in 100-mL industrial wastewater ~~in~~ at various conditions. Cell density in (—●—) wastewater (control), (—□—) wastewater + *B. cereus* NI, and (—■—) wastewater + *B. cereus* NI + mineral salts medium supplemented with 0.1% (w/v) yeast extract. BPA removal efficiency in (—●—) wastewater (control), (—□—) wastewater + *B. cereus* NI, and (—■—) wastewater + *B. cereus* NI + mineral salts medium supplemented with 0.1% (w/v) yeast extract. Bars indicate the mean ± SD of three replicates.

Figure 4 Comparison of BPA removal efficiency of *D. sanderiana*-endophyte *P. dispersa* with *B. cereus* NI in 500-mL industrial wastewater at various conditions: (●) wastewater, (○) wastewater + *B. cereus* NI, (■) wastewater + plant, and (□) wastewater + *B. cereus* NI + plant. Bars? Average values?

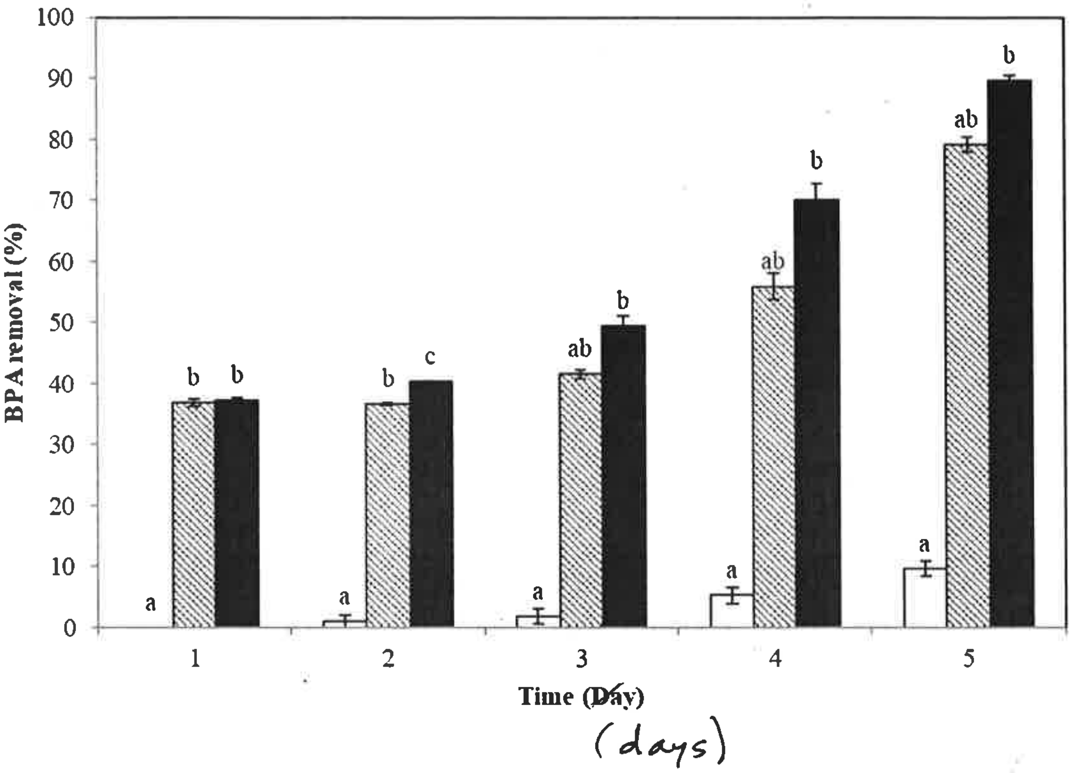

100
90
80
70
60
50
40
30
20
10
0
BPA removal (%)
a
b
b
a
b
c
a
ab
b
a
ab
b
a
ab
b
1
2
3
4
5
Time (Day)
(days)

Figure 1

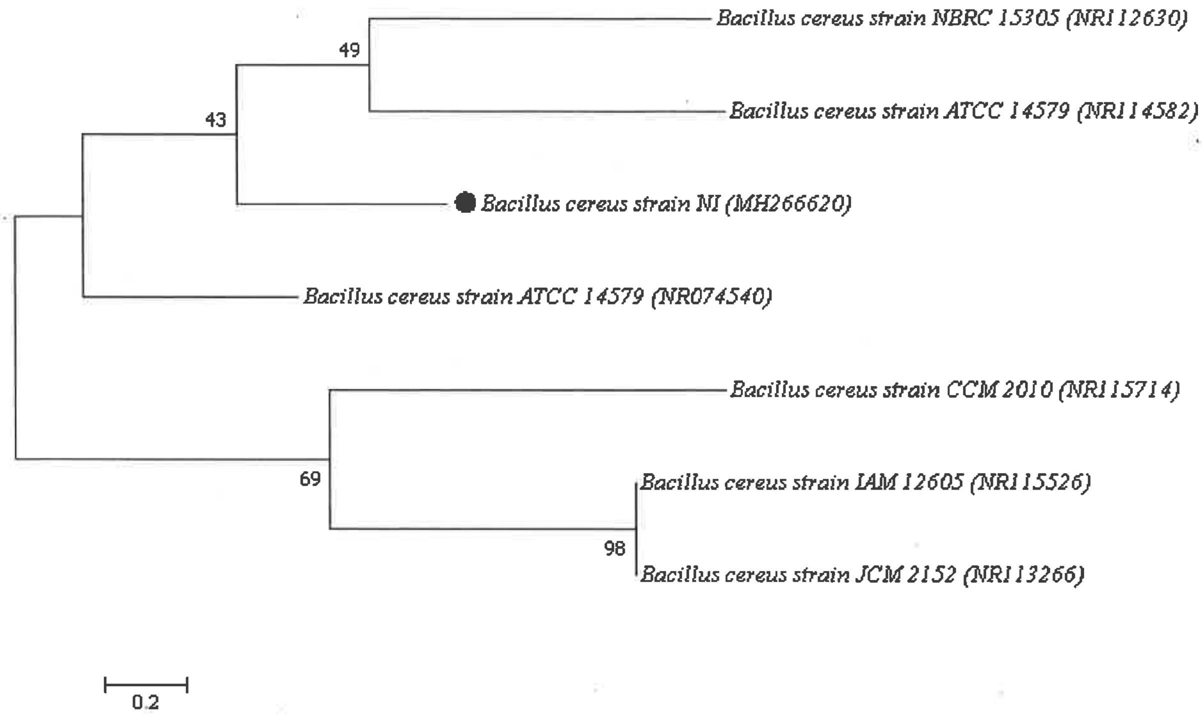
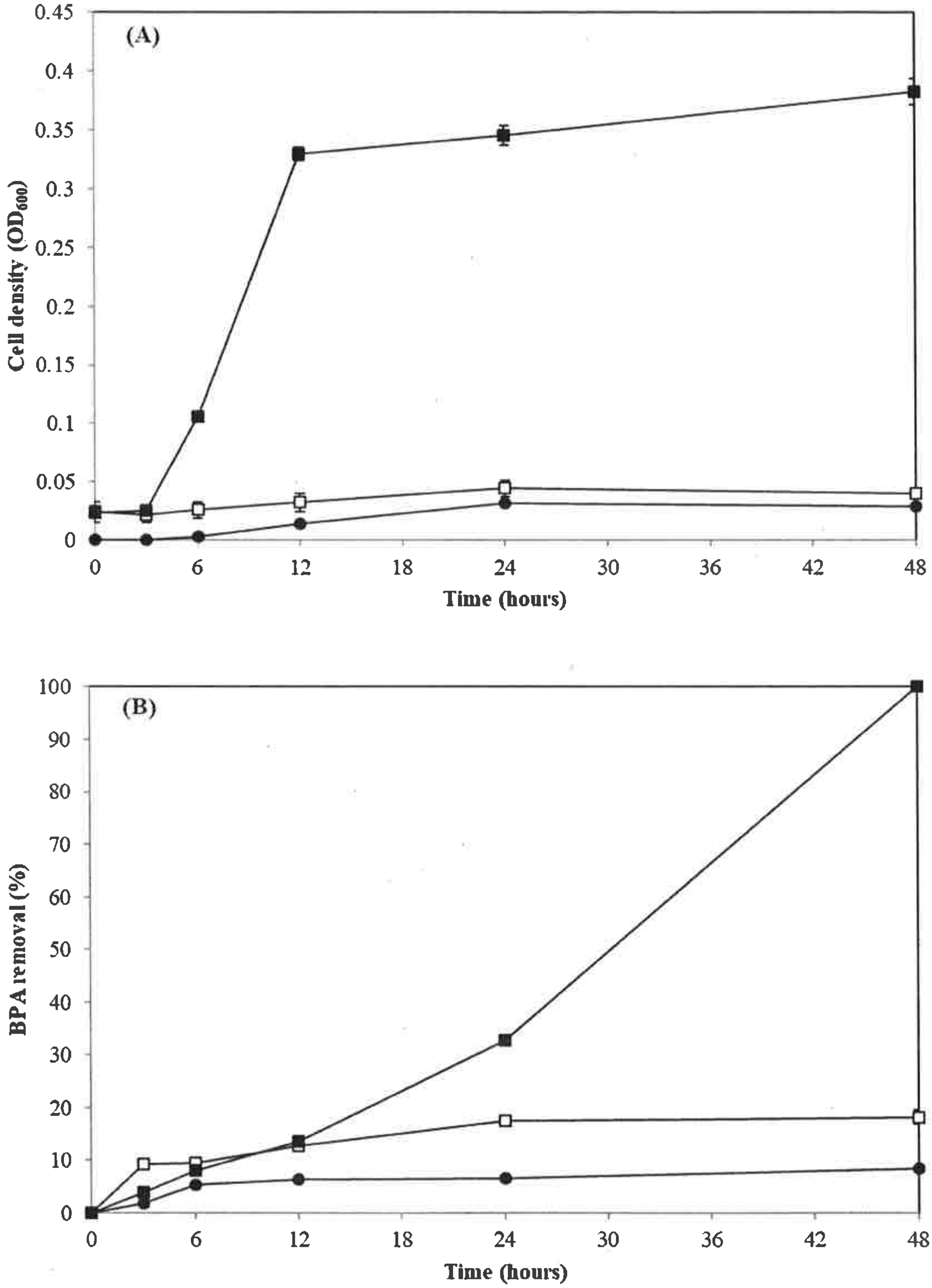

Bacillus cereus strain NBRC 15305 (NR112630)
49
Bacillus cereus strain ATCC 14579 (NR114582)
43
Bacillus cereus strain NI (MH266620)
Bacillus cereus strain ATCC 14579 (NR074540)
Bacillus cereus strain CCM 2010 (NR115714)
69
Bacillus cereus strain IAM 12605 (NR115526)
98
Bacillus cereus strain JCM 2152 (NR113266)
0.2

Figure 2



(A)
Cell density (OD_{600})
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
6
12
18
24
30
36
42
48
Time (hours)
(B)
BPA removal (%)
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
Time (hours)

Figure 3

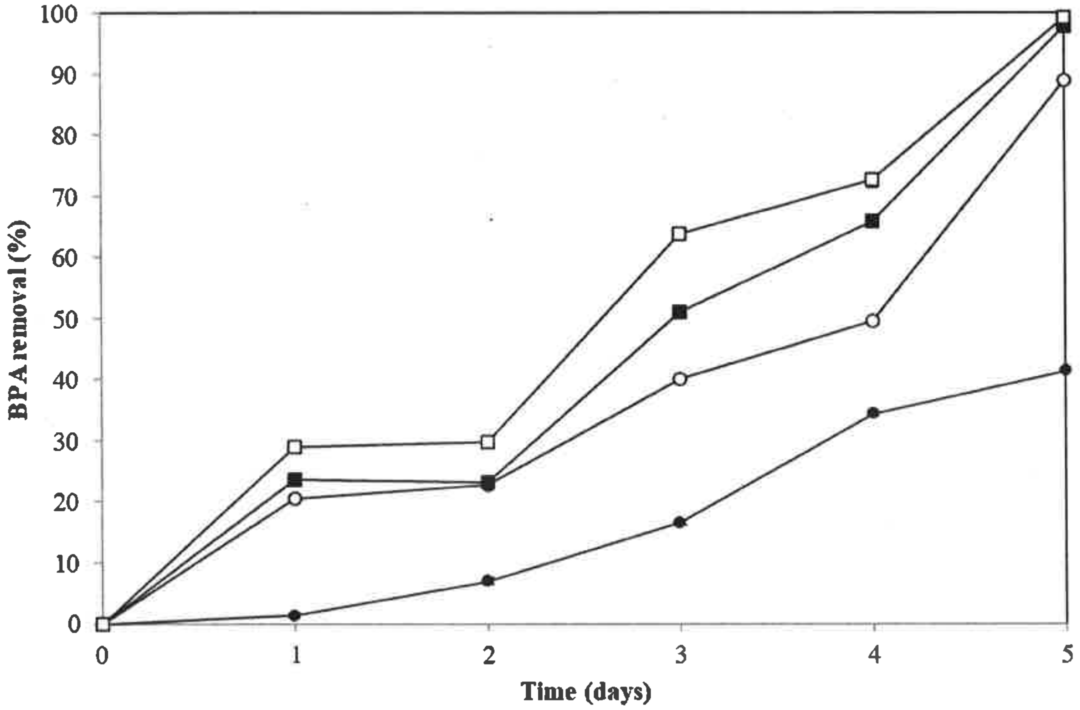


Figure 4

Table 1 pH, COD, BOD, EC, salinity, and TDS in a plastic industrial wastewater under various conditions after 5 days of phytoremediation.

Treatment/ Parameter	pH	COD (mg/L)	BOD (mg/L)	TDS (mg/L)	Conductivity (µS/cm)	Salinity (ppt)
Initial wastewater	9.32 ± 0.11^a	809.05 ± 37.71^a	107.50 ± 3.53^b	$56{,}379 \pm 352.52^c$	48.21 ± 0.53^c	46.00 ± 0.00^b
Wastewater	9.30 ± 0.04^a	793.77 ± 34.38^a	45.00 ± 0.00^a	$53{,}245 \pm 208.65^b$	43.73 ± 0.33^b	41.33 ± 3.05^a
Un ~~Non-~~inoculated plant	8.91 ± 0.03^b	740.74 ± 61.72^a	38.75 ± 1.76^a	$50{,}327 \pm 1{,}167.73^a$	39.36 ± 1.74^a	40.67 ± 1.15^a
Inoculated plant	8.84 ± 0.10^b	732.51 ± 31.07^a	30.00 ± 21.00^a	$48{,}670 \pm 1{,}755.74^a$	36.92 ± 2.59^a	39.33 ± 1.52^a

Data are means ± SD (n = 3). Values in the same column with the same letters are not significantly different (α = 0.05). Un ~~Non~~-inoculated plant = plant without ~~the~~ endophytic *P. dispersa*; Inoculated plant = plant inoculated with endophytic *P. dispersa*.